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Searching for an effective, safe and universal anti-HIV vaccine

Finding the answer in just one short peptide

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We explore the pentapeptide overlapping between human immunodeficiency virus (HIV) proteins and the human proteome. Our intent was to define viral peptides to be used in vaccines effective against different HIV strains, vaccines that are able to overcome the difficulties posed by the tendency of HIV to mutate, and that are also exempt from harmful collateral cross-reactions, as well as being repeatedly administrable to the global population. Analysis of HIV-1 envelope glycoprotein 160 (Env gp160) sequences revealed a set of 15 pentapeptides highly conserved among a number of retroviral sequences, and absent in the human proteome, thus representing unique molecular retroviral signatures. Use of these short viral peptide modules may represent the first concrete step toward the goal of a universal, safe and effective anti-HIV vaccine.

Introduction

Both ongoing, strenuous efforts and discouraging results have characterized the more than two-decade long search for an HIV vaccine.[1-5] The main obstacles to effective anti-HIV immunotherapies are: (1) the tendency of human retroviruses to rapidly mutate, resulting in a high amino acid sequence variability[6,7] and (2) the concern of inducing collateral autoimmune phenomena through responses cross-reactive with the host proteome. Cardiolipin polyspecific autoreactivity by two broadly neutralizing HIV-1 monoclonal antibodies is just such an example of potential cross-reactivity.[8] In point of fact, using sequence-to-sequence peptide matching to analyze the peptide commonality between HIV and human proteins, we found that HIV pentapeptides are widely, repeatedly and intensively represented in human proteins, with only a relatively limited number of viral pentameric fragments (about 10%) being unique to the retroviruses.[9-11] The extensive peptide identity pattern between HIV-1 and humans equates to a high risk of cross-reactivity in the course of immune anti-HIV-1 responses, and possibly explains the link between HIV-1 infection and AIDS.[11]

With the ambitious aim of developing a safe, effective and universal anti-HIV-1 vaccine, the present study explores the HIV-1 Env gp160 primary sequence and describes a viral pentapeptide set highly conserved among HIV sequences from different strains, and not represented in the human proteome. We propose use of such unique viral peptide signatures for designing global anti-HIV-1 vaccines that are both efficacious and exempt from harmful collateral cross-reactions.

Results

In this study we analyzed four HIV-1 Env gp160 primary sequences. Env gp160 seems to contribute to T-cell depletion during HIV-1 infection and allows rapid transcytosis of the virus through CD4 negative cells such as the simple epithelial monolayers of the intestinal, rectal and endocervical epithelial barriers.[12] Also, it seems involved in cell-to-cell spreading of HIV-1.[13] Therefore, finding vaccines able to specifically target Env gp160 might inhibit HIV-1 infection and the related immunodeficiency. Moreover, HIV-1 Env gp160 was chosen as an experimental model for this study because of its high level of sequence variability.[14]

The four HIV-1 Env gp160 primary sequences were derived, respectively, from (1) a major HIV-1 lineage isolated in France (X01762, group M, subtype B, isolate BH10); (2 and 3) two minor variants found in Yaounde, the capital city of Cameroon (AJ291719 and AJ291720);[15,16] and (4) an infectious molecular clone derived from a Spanish HIV-1 isolate (AJ006287, subtype B).[17] In this way, we tried to compare common and rare HIV-1 sequences as well as HIV-1 sequences from different geographical areas.

Pentapeptides were used as scanning probes since modules of five to six amino acids represent minimal determinants involved in B- and T-cell immune recognition.[18] As a matter of fact, already in 1939 Landsteiner and van der Scheer demonstrated that "antibodies may be formed which are specific for peptide chains consisting of five amino acid residues".[19]

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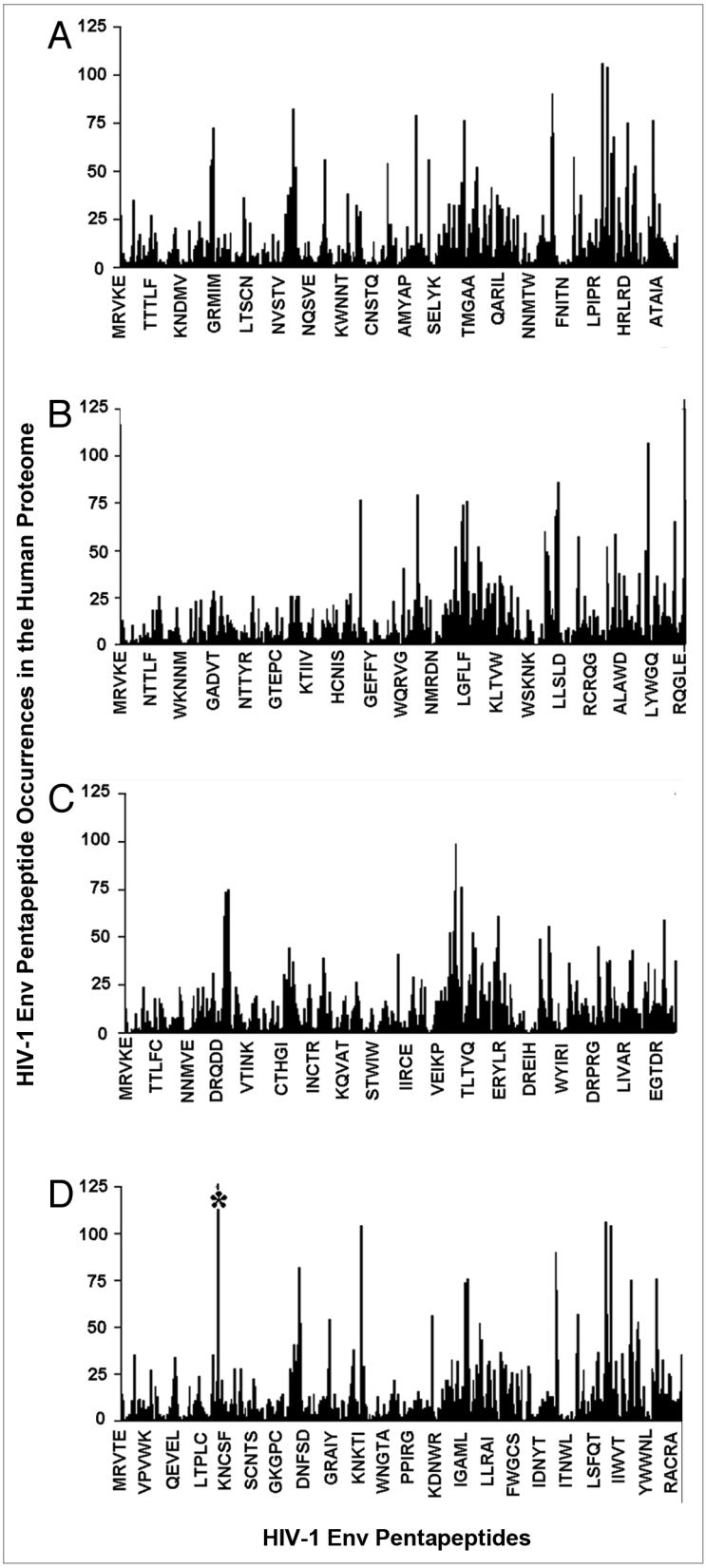

Figure 1. Pentapeptide identity profile of four Env gp160 sequences to the human proteome. (A–D) refer to Env gp160 UniProtKB/Swiss-Prot accession: (A) P03375; (B) Q90DZ7; (C) Q8UMG1; and (D) O93024. (D) the asterisk indicates the viral SSSGG pentapeptide, which is represented more than 200 times in the human proteome.

Also, Landsteiner and van der Scheer reported that antisera against the pentapeptide Gly-Gly-Gly-Gly-Leu distinguished between Gly-Gly-Gly-Gly-Leu and Gly-Gly-Leu-Gly-Gly, thus indicating that anti-pentapeptide antibodies show a high degree of specificity. This foundational paper has been followed by a number of reports, all congruent in defining pentapeptides as the minimum chain length for immune recognition.[20-31]

Definition of unique HIV-1 Env gp160 pentapeptides for non cross-reactive vaccine formulations. As advocated by Kanduc,[32-37] only vaccines based on unique antigenic peptides might guarantee no/low cross-reactivity and the highest specificity. Hence, as a first step in this study, we searched for pentapeptides unique to the viral proteins. **Figure 1** illustrates the pentapeptide identity profile of the four HIV-1 Env gp160 sequences versus the human proteome, with the x-axis reporting viral pentapeptides sequentially overlapping by four residues, and the y-axis indicating the numbers of matches of each viral pentapeptide to the human proteome. **Figure 1** clearly documents that, firstly, the pentapeptide identity profile to the human proteome has a wave pattern in the four viral proteins, with high similarity sequence areas alternating with those of low similarity. Secondly, almost all of the pentapeptide blocks forming the HIV-1 Env gp160 sequences are also repeatedly present in human proteins; thus, only a limited number of pentamers are unique to the viral proteins. A high degree of peptide matching persists even when hexapeptide motifs were used as probes for sequence identity scanning (data not shown).

Quantification of the pentapeptide identity platform between the HIV-1 Env gp160 sequences and the human proteome is reported in **Table 1**. Around 90% of the pentapeptides forming the HIV-1 Env gp160 sequences also occur in the human proteome. Moreover, the shared HIV-1 Env gp160 pentapeptides are dispersed throughout the human proteome. Indeed, the mean number of times each shared 5-mer from the Env gp160 sequences occurs in the whole human proteome is ~11 (i.e., the number of multiple occurrences divided the number of shared viral pentapeptides). According to **Figure 1** and **Table 1**, a vaccine formulation using an entire HIV-1 Env gp160 as an antigen would produce a risk of cross-reactivity with human proteins amounting to thousands of hits. Only about 10% of viral pentapeptides do not have a match in the human proteome; these represent, therefore, molecular signatures of the retroviral antigens. By being unique to the HIV-1 Env gp160 sequences, these zero similarity pentapeptides constitute peptide sets usable in pharmaceutical vaccine formulations theoretically devoid of any cross-reactivity potential.

Definition of conserved HIV-1 Env gp160 pentapeptides for worldwide effective vaccine formulations. HIV-1 is extraordinarily variable,[38] and this variability represents a major obstacle to AIDS vaccine development. To solve the diversity problem, country- and isolate-specific

Table 1. Pentapeptide overlapping between four HIV-1 Env gp160 proteins and the human proteome

Env gp160[1]	HIV-1[2]	Env gp160 pentapeptides:			
		Total	Shared with human proteins	Occurrences in human proteins[3]	Unique to the viral protein
P03375	X01762	852	768	8907	84
Q90DZ7	AJ291719	851	768	8424	83
Q8UMG1	AJ291720	845	761	8229	84
O93024	AJ006287	858	775	8940	83

[1]Env gp160 given as UniProtKB/Swiss-Prot accession number. [2]HIV-1 given as GenBank accession number. [3]Number of total Env gp160 pentapeptide occurrences in the human proteome, including multiple occurrences.

vaccines can be considered, but even these solutions appear of doubtful efficacy in light of the extreme polymorphism shown by HIV-1.[39] Moreover, they would add a further economic burden to the population to be vaccinated.

With the aim of identifying consensus sequences to be used in globally-effective vaccines, we analyzed HIV-1 Env gp160 unique pentapeptides for conserved sequences. That is, the four HIV-1 Env gp160 sequences were aligned and the common unique viral 5-mers were singled out.

As visualized in **Figure 2**, alignment of the four HIV-1 Env gp160 sequences reveals a high level of conservation on the whole. Indeed, only 15 pentapeptides are both unique to the virus and possess a conserved sequence. These pentapeptides are: GMLMI, WVTVY, TLFCA, LFCAS, LKPCV, KPCVK, IPIHY, PIHYC, HYCAP, YCAPA, SFNCG, NCGGE, GEFFY, VWGIK and HIPRR.

These 15 unique viral pentapeptides, absent in the human proteome and common to the four Env gp160, were used to scan the PIR database of retroviral proteomes. The purpose was to ascertain whether these pentapeptides were also present in Env gp160s from other HIV strain/group/subtype isolates. An example of the data is reported in **Table 2**, which describes the HIVs hosting the pentapeptide KPCVK. The HIV lists relative to the remaining 14 pentapeptides are reported in **Supplemental Table 3**.

The data in **Table 2 and Supplemental Table 3** are striking. Indeed, **Table 2** documents that antibodies against a small peptide module formed by only five amino acid residues, i.e., the pentapeptide KPCVK, would have the potential to target 57 HIV isolates from different strains (HIV-1 and HIV-2), groups (M, O and N as well as A and B) and numerous isolates of diverse geographical origins. Also, and most importantly, antibodies specific for such a small peptide module would have no cross-reactivity, i.e., they would not induce

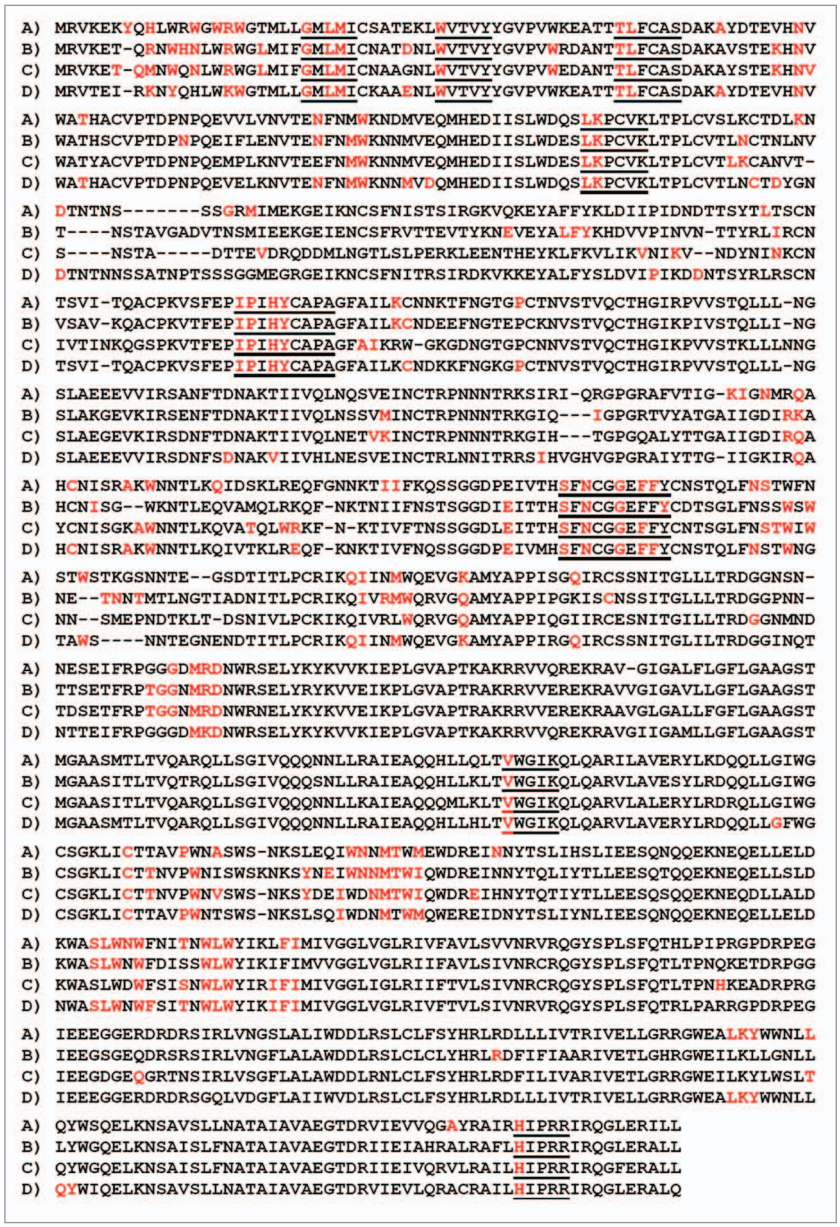

Figure 2. Sequence alignment of the four Env gp160 sequences under study. First residue of pentapeptides unique to the viral sequences and not found in human proteins is given red. Pentapeptides unique to the viral sequences and conserved among the four Env gp160 sequences are underlined. Env gp160 sequences refer to UniProtKB/Swiss-Prot accession: (A) P03375; (B) Q90DZ7; (C) Q8UMG1; and (D) O93024.

Table 2. HIVs hosting the pentapeptide KPCVK

Type	Group	Subtype	Isolate	Taxonomy ID
1	M	J	SE9173	388904
1	M	J	SE9280	388905
1	M	F1	VI850	388813
1	M	K	97ZR-EQTB11	388907
1	M	F2	MP255	388815
1	M	F2	MP257	388823
1	N	-	YBF106	388819
2	B	-	EHO	388821
2	B	-	UC1	388822
1	M	C	ETH2220	388796
2	A	-	KR	73484
1	M	B	LW123	82834
1	M	B	YU-2	362651
2	A	-	ST/24.1C#2	31681
1	M	B	WMJ1	31678
1	M	B	KB-1/ETR	36375
2	A	-	CAM2	11715
1	M	B	OYI	11699
2	A	-	ST	11721
1	M	B	JRCSF	11688
1	M	B	MFA	11704
1	M	B	PIRSF162	11691
1	M	B	PIRSF33	11690
1	M	D	NDK	11695
2	A	-	BEN	11714
2	A	-	Ghana-1	11717
2	A	-	D194	11713
2	B	-	D205	11716
1	M	U	Z3	11680
1	M	B	NY5	11698
1	M	B	JH32	11694
1	M	B	BRVA	11693
1	M	D	Z2/CDC-Z34	11683
2	A	-	SBLISY	11718
2	A	-	NIH-Z	11719
1	M	D	Z84	11681
1	M	A	Z321	11692
1	M	B	WMJ22	11705
1	M	B	CDC-451	11687
1	M	B	SC	11702
1	M	B	MN	11696
1	M	B	HXB3	11707
1	M	A	MAL	11697
1	M	B	BH8	11684
1	M	D	ELI	11689
1	M	D	Z6	11708
1	M	B	RF/HAT3	11701

Table 2. HIVs hosting the pentapeptide KPCVK

Type	Group	Subtype	Isolate	Taxonomy ID
1	M	B	HXB2	11706
2	A	-	ROD	11720
1	M	B	ARV2/PIRSF2	11685
1	M	B	BRU/LAI	11686
1	M	B	BH10	11678
1	N	-	YBF30	388818
1	M	F1	93BR020	388814
1	M	H	90CF056	388826
1	M	G	92NG083	388825
1	M	C	92BR025	388812

autoimmune reactions since this sequence is not represented in the human proteome.

Discussion

The search for an HIV-1 vaccine remains a challenge for science and medicine. A number of anti-HIV-1 vaccines, such as AIDSVAX, AIDSVAX B/B, AIDSVAX B/E and HIV gp120, did not pass the final test.[40] Recently, and representing a substantial investment of money and effort, the STEP and Phambili trials were launched. The immunotherapeutic approach of the STEP and Phambili trials was based on a complex replication-defective adenovirus type 5 (Ad5) MRK gag/pol/nef HIV vaccine, and hopes for its success were high.[41] However, the trials failed and were halted at the first interim analysis due to an absence of an even minimally positive effect. Indeed, increased susceptibility to HIV-1 was found in men receiving the vaccine.[42] The question—"Is an HIV vaccine possible?"[43]—thus appears to be legitimate.

The present work proposes a possible answer to this question. Using only four HIV sequences, a major isolate and three minor variants, we identified a minimal determinant—the pentapeptide KPCVK—present in 57 different HIV sequences. That is, antibodies designed to hit the pentapeptide KPCVK would target a large range of retroviral sequences, thus opening the way to a global approach to the prevention and cure of HIV infection. Hence, it is logical to postulate that applying this rationale and using the data illustrated in **Supplemental Table 3**, a universal anti-HIV weapon may be developed using only a small number of peptides. Likewise, this methodological approach might also be applied to other viral proteins. Of special interest, it has to be noted that many of the 15 pentapeptides absent in the human proteome and shared by numerous HIVs are also present in simian immunodeficiency viruses (SIVs). For example, the pentapeptide KPCVK is found in SIV-wrc Pbt-05GM-X02 (Tax ID: 498715), SIV-mnd 1 (Tax ID: 358183), SIV African green monkey vervet (isolate AGM3) (Tax ID: 11730), SIV 17E-Fr (Tax ID: 160753), SIVmac (Tax ID: 11711). Clearly, utilization of pentapeptides shared by SIVs and HIVs would imply a fast transfer of results from animal models to clinical human trials in vaccine testing and evaluation, with a paramount gain in time and

economical investment. In any case, and most importantly, the eventual vaccine preparations would be safe. Being based on peptide sequences not present in the human proteome, these vaccines will probably be free of cross-reactivity,[32-37] therefore nullifying the risk of autoimmune collateral events, even following repeated administration. In conclusion, global and safe protection from HIV infection appears to be at hand.

Methods

UniProtKB/Swiss-Prot accession of the analyzed Env gp160 sequences are: P03375, from HIV-1 sequence X01762, BH10 group M subtype B, 11678; Q90DZ7, from AJ291719, a minor variant from Yaounde, the capital city of Cameroon;[15,16] Q8UMG1 from AJ291720, a minor variant from Yaounde;[15,16] O93024 from HIV-1 AJ006287, subtype B, an infectious molecular clone derived from a Spanish HIV-1 isolate.[17] The sequences were downloaded from www.uniprot.org.[44] Each viral sequence was dissected into consecutive pentapeptides offset by one amino acid residue. Pentapeptides were then utilized as probes to scan the human and retroviral proteomes for exact matches using the Protein Information Resource perfect match program (pir.georgetown.edu/pirwww/search/peptide.shtml).[45] Sequence alignment was performed using the T-COFFEE Software (www.tcoffee.org).[46]

Authors' Contributions

G.L. and A.S. performed the computational analyses. D.K. proposed the original idea, interpreted the data, developed the research project and wrote the manuscript. All authors discussed the results, and revised and commented on the manuscript.

Note

Supplemental materials can be found at:
www.landesbioscience.com/journals/selfnonself/article/14762

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